REMARKS

Support for new claims 53 and 54 can be found in original claims 45 and 52, respectively. Claims 1-54 are pending in the application; claims 1-24, 42 and 43 have been withdrawn and claims 25-41 and 44-54 are under consideration.

On page 2 of the Office Action, the examiner has objected to the specification on the basis that page 15 contains a hyperlink. This objection has been obviated by the amendment above to the specification.

Claims 25-41 and 44-52 have been rejected under 35 U.S.C. §112, second paragraph, as indefinite in their recitation of "3' terminus" and "5' terminus." The examiner asserted that it is unclear whether "3' terminus or 5' terminus" refers to the first nucleotide on the 3' or 5' end of the polynucleotide or whether it includes any nucleotide that has another nucleotide 5' to the 3' terminus or 3' to the 5' terminus. This rejection is traversed.

Applicants respectfully submit that the terms "terminus" and "termini" are clear and well-known in the art and are defined in accordance with conventional understanding in the present application. On page 11, lines 8-11, of the specification, the termini are also called "end regions." This is further supported by a more detailed explanation of the term "terminus" on page 13, lines 24-29, of the specification:

> For the purposes of the present application, the terms 5'-terminus, 5'-end and 5'-tag are equivalent to each other and can be used interchangeably. In the same way, the terms 3'- terminus, 3'-end and 3'-tag are equivalent to each other and can be used interchangeably. In an original nucleic acid molecule or portion inside a nucleic acid molecule that one intends to reduce or represent, each of the 5'-end and 3'-end represents a region or portion most closer to the extremity and most far from the middle region or portion most close to the extremity and most far from the middle region of the molecule.

In fact, the definition of the term "terminus" provided in the application is in agreement with the examiner's own interpretation at page 6, second paragraph, of the outstanding

Office Action, where he stated that he interpreted "5' or 3' terminus of a polynucleotide as any fragment or whole piece of a polynucleotide that contains the actual 5' or 3' terminus of the polynucleotide."

It is clear from the specification that a terminus is more than a single nucleotide. As such, reference to a "terminus" does not strictly refer to a single nucleotide.

Claims 45-47 and 52 also have been rejected under this same paragraph of Section 112 of the patent statute on the basis that the recitation "...wherein each adapter includes at least one restriction site and wherein each adapter includes at least a first restriction site which is an asymmetric restriction site and at least a second restriction site." The examiner asserted that it was unclear how each adapter could have one restriction site but have a first restriction site and a second restriction site. Applicants submit that this rejection has been obviated by the amendments to claims 45 and 52.

The examiner has rejected claims 25-35, 38 and 43-45 (as claim 43 has been withdrawn, Applicants assume the rejection applies to claims 25-35, 38 and 44-45) under 35 U.S.C. §102(b) as anticipated by U.S. Patent 6,383,743, issued to Kinsler et al., hereinafter referred to as the '743 patent. The examiner characterized the '743 patent as teaching a method of creating ditags where cDNA is cleaved by an anchoring enzyme, fragments containing the 3' or 5' ends of the cDNA are isolated and ligated with linkers, the fragments are cleaved again by an asymmetric restriction enzyme with a recognition site within the linker, and the resulting fragments are ligated back together and can be cleaved again by the anchoring enzyme for concatenation. This rejection is traversed.

Applicants note that in setting out this rejection, the examiner stated, in the last full sentence on page 6 of the Office Action, that the '743 patent teaches that "subsequent ligation will ligate fragments from different genes together but will also ligate the 3' and 5' termini of the complimentary strands of the same polynucleotide together flanked by two adapters (claims 25 and 26)." Applicants have been unable to find such a disclosure in the '743 patent. Applicants therefore submit that claims 25

and 26, and the claims dependent from them, are novel over the teachings of the '743 patent.

Applicants note the '743 patent provides at column 4, lines 21-25, that each ditag represents two defined nucleotide sequences of at least one transcript, representative of at least one gene. The term "defined" nucleotide sequence is said to refer to a nucleotide sequence derived from either the 5' or 3' terminus of a transcript (column 4, lines 39-41, of the '743 patent). This indicates that the 5' end and the 3' end are not from the same gene, contrary to the situation in the method of claims 25 and 26 of the present application. In claims 25 and 26, the claimed methods provide for the splicing of the 5' and 3' ends of the same nucleic acid molecule.

Furthermore, at column 2, lines 45-46, and column 4, lines 32-32 of the '743 patent, it is stated that the method therein is one of random pairing. It also involves random dimerization of tags (column 3, lines 45-46 of the '743 patent). From these passages, it is clear that the '743 patent does not disclose or suggest the preparation of a ditag from the same nucleic acid molecule. Accordingly, the reference does not disclose the invention of claims 25 or 26 or the claims dependent from them.

Claims 25-30, 33-35. 38 and 44-48 have been rejected under 35 U.S.C. § 102(b) as anticipated by U.S. Published Application 2002/0025561 (hereinafter the '561 application), filed by Hodgson. The examiner characterized the reference as teaching a method of assembling DNA molecules which includes isolating the 3' and 5' terminus of a polynucleotide that has been phosphorylated, interpreted as an adapter, into a vector that has two adapters having type IIS restriction endonuclease sites, including *Sap*1, cutting the DNA out of the vector with the appropriated restriction endonuclease, and ligating the DNA with other DNAs into the vector to link the 5' and 3' terminus of the original polynucleotide. This rejection is traversed.

Applicants submit that, contrary to the examiner's assertion regarding the '561 application noted in the preceding paragraph, the reference does not disclose that the 3' and 5' termini of a polynucleotide were isolated, joined and inserted into a vector. Applicants respectfully request that the examiner specifically point out the passages in

the '561 application that teach where the 5' and 3' termini of a nucleic acid molecule are not removed but rather are retained as taught and claimed in the present application.

Applicants submit that from a reading of the '561 application, especially Figure 1, it is apparent that the insert comprises the polynucleotide with part of its 3' and 5' termini removed by the *Sap*1 enzyme in order to release the insert for subsequent ligation with other inserts. It is clear that the insert fragments that actually are annealed and ligated (i.e., concatenated) actually are the original DNA molecules minus their 3' and 5' termini. Thus, it is clear from the '561 application that the method taught therein is the opposite of the method claimed in the present application. The '561 application is addressed to assemble inserted fragments, minus their 3' and 5' ends (See, in particular Figure 1C of the reference), while the claimed invention is addressed to splice the 3' and 5' termini of a nucleic acid molecule into a ditag. The presently claimed invention thus is novel over the disclosures of the '561 application.

Claim 35 has been rejected under 35 U.S.C. §102(a) as unpatentable over the '743 patent in view of the New England Biolabs 2000/2001 Catalog. The examiner characterized the '743 patent as he had in relying upon the reference in the §102 rejection discussed above. He noted that the '743 patent does not teach a method of ditag construction in which the type II restriction endonuclease is *Mme*I, but he asserted that the secondary reference teaches that this endonuclease is an asymmetric restriction enzyme that cleaves 18-20 bp away from its recognition site and that it would have been obvious to one of ordinary skill in the art to use *Mme*I in the method of ditag construction taught in the '743 patent. This rejection is traversed.

The shortcomings of the '743 patent have been discussed above, and that discussion is equally applicable to the present rejection. The '743 patent teaches the random ligation of 3' and 5' termini from different polynucleotides, rather than the splicing of the 5' and 3' ends of the same nucleic acid molecule. Furthermore, there is no indication in the '743 patent that *Mme*I could be used as the type II restriction endonuclease. The option of using that endonuclease may have been available, but there would have been no expectation that the use of *Mme*I would work successfully in

the present invention. The secondary reference does not compensate for the deficiencies of the primary reference, and the two references do not suggest the claimed invention: even if the teachings of the two references were combined, the resultant product would be very different from that claimed. The references thus do not suggest the invention set forth in claim 35.

Claim 36 has been rejected under 35 U.S.C. §103(a) as unpatentable over the '743 patent in view of Belfort et al., *Nucleic Acids Research* 25:3379-3388 (1997)). The examiner characterized the '743 patent as he had previously in relying upon the reference in other rejections discussed above. He acknowledged that the '743 patent does not teach a method of preparing ditags specifically where the ditag is flanked with adapters containing homing endonuclease asymmetric restriction sites, but he asserted that the references discloses an embodiment in which the anchoring endonuclease used in the ditag construction rarely cuts cDNA such that few or no cDNA representing abundant transcripts are cleaved. The secondary reference was cited as teaching that homing restriction enzymes are rare cutting enzymes with asymmetric restriction sites. He asserted that it therefore would have been obvious to using homing restriction enzymes in the adapters flanking the ditags taught by the '743 patent in view of the disclosures in the *N.A.R.* reference. This rejection is traversed.

As Applicants previously have pointed out, the '743 patent does not teach the ligation of the 3' and 5' termini of the same polynucleotide but, rather, the random ligation of 3' and 5' ends of different polynucleotides. Further, the '743 patent provides that random dimerization is a necessary procedure for reducing bias (See column 3, lines 45-46). Thus, according to these teachings, the ligation of the 3' and 5' termini of the same polynucleotide is to be avoided. Further, unlike the method of the present invention, the '743 patent does not teach any step to select and/or ensure the ligation of the 3' and 5' termini of the same polynucleotide and/or that only such polynucleotides are to be analyzed. The teachings of the secondary reference do not compensate for the deficiencies of the primary reference. There is no motivation provided to combine the teachings of the two references, but even if one were to do so, the resultant product

would be very different from that obtained by the method of claim 36 of the present application. Applicants thus submit that the subject matter of claim 36 is not rendered obvious by the combined teachings of the two cited references.

Claims 39-43 have been rejected under 35 U.S.C. §102(a) as obvious over the '743 patent in view of Saha et al., *Nature Biotechnology* 19:508-512(2002). The examiner acknowledged that the primary reference does not teach a method of ditag construction and analysis which specifically comprises mapping each of the two tags of the ditag on the genome and further defining the structural region of the corresponding gene on the genome map; or comparing the ditag with genome map or a database, detecting matching 5' or 3' termini on the genome map but detecting no match on one or more gene databases and further recovering the newly discovered gene; or sequencing a ditag from a full length cDNA library and recovering the full-length cDNA corresponding to the ditag. The examiner asserted, however, that these steps were obvious in view of the teachings of the cited secondary reference, which was characterized as teaching that ditags from a cDNA library can be matched to genomic sequences which allow precise localization of tags in the genome and that the comparison of tag locations with position of previously annotated genes can provide expression evidence for predicted genes and identify previously uncharacterized genes. This rejection is traversed.

Neither of the cited references teaches or suggests the use of ditags in any method of genome mapping, gene discovery or recovery of full-length cDNA wherein the 5' terminus and the 3' terminus of the same nucleic acid molecule or fragment thereof are linked or joined. There is no motivation provided for one of skill in the art to combine the teachings of the two references, and even if one were to do so, one would not obtain the results obtainable by the present invention as this key feature of the present invention is neither disclosed or suggested by the cited references. Accordingly, claims 39-42 of the present application are patentable over the cited art.

Claims 46-49 have been rejected under 35 U.S.C. §103(a) as unpatentable over the '743 patent in view of the New England Biolabs 2000/2001 Catalog. The examiner

acknowledged that the '743 patent does not teach ditags flanked by adapters with asymmetric and second restriction sited inserted into vectors specifically where the vector backbone does not comprise the restrictions sites of the adapters. He asserted, however, that one of ordinary skill in the art would realize that having such restriction sites would significantly compromise the efficiency of ligating the ditags into the vector as more vector fragments would need to be isolated and included in the ligation reaction and that it therefore would have been obvious to improve the method of ditag construction as taught in the '743 patent such that ditags flanked by adapters with asymmetric and second restriction sites are inserted into vectors where the vector backbone does not comprise the restriction sites of the adapters. The examiner further acknowledged that the '743 patent does not teach a method of ditag construction where ditags flanked by asymmetric and second restriction sites inserted into vectors specifically are inserted into vectors where the vector backbone does not comprise *Mme*I sites of the adapters. He asserted, however, that in view of the description of the *Mme*I enzyme in the N.E.B. catalog that it would have been obvious to use that enzyme in the method of ditag construction. This rejection is traversed.

All of the previous comments regarding the deficiencies of the '743 patent are applicable to the present rejection as well. Claims 46-49 are dependent claims, and as has been discussed, the '743 patent does not anticipate or render obvious the invention set forth in the claims from which claims 46-49 depend. Furthermore, claims 46-49 provide that the backbone of the vector does not comprise the asymmetric restriction site or the second restriction site. The use of such a specific vector is neither disclosed nor suggested by the '743 patent. In fact, in that reference the purpose of the vector is for the sequencing of ditags, wherein in the claimed methods of the present invention the purpose of the vector having a backbone which does not comprise the asymmetric restriction site or the second restriction site is to ensure that the 5' and 3' termini come from the same nucleic acid molecule. The examiner has asserted that one of skill in the art would want to use a vector which has a backbone that does not comprise the asymmetric restriction site or the second restriction site in order to increase the

efficiency of the ligation of the ditags to reduce the number of fragments being produced. However, as pointed out above, this is not the case in the present invention and the purpose of using such a vector is completely different. It thus would not have been obvious to use a vector having a backbone which does not comprise the asymmetric restriction ste or the second restriction site. The cited secondary reference does not compensate for the deficiencies of the primary reference, and so the combined teachings of the two references does not render obvious the subject matter of claims 46-49.

Claim 37 has been rejected under 35U.S.C. §103(a) as obvious over the '561 application previously cited above. The examiner acknowledged that the reference does not teach a method of ligating the 3' and 5' termini of a polynucleotide where the polynucleotide specifically is a cDNA molecule, but he asserted that the reference does teach the assembly of several types of polynucleotides, including exons, and that one of ordinary skill in the art would recognize readily that full-length cDNA can be used to represent the exon of a one exon gene, such that it would have been obvious to use cDNAs in the method of assembling DNA molecules as taught by the '561 application. This rejection is traversed.

The shortcomings of the '561 application have been discussed above in connection with other claims, including the claim from which claims 37 depends, and that discussion is equally applicable to the present rejection. In addition, Applicants submit that the examiner's interpretation of the '561 application as teaching the isolation of "the 3' and 5' terminus of a polynucleotide that has been phosphorylated, interpreted as an adapter, into a vector..." is misguided; no such teaching is apparent to the Applicants. From a reading of the '561 application, particularly figure 1B, it is apparent that the insert comprises the polynucleotide with part of its 3' and 5' termini removed by the *Sap*1 enzyme in order to release the insert for subsequent ligation with other inserts. It is clear that the insert fragments that actually are annealed and ligated (i.e., concatenated) actually are the original DNA molecules minus their 3' and 5' termini. Applicants request that the examiner specifically point out the passages in the '561

application that teach the method of the present invention wherein the 5' and 3' ends of a nucleic acid molecule are retained, rather than being removed.

Claim 49 of the application has been rejected as unpatentable under 35 U.S.C. §103(a) as obvious over the teachings of the '561 application in view of Tucholski, *Gene* 157:87-92 (1995). The examiner acknowledged that the primary reference does not teach a method of assembling DNA molecules specifically where the adapters used comprise *Mme*I sites, but he asserted that the reference teaches that appropriate class II restriction enzymes can be used in the adapters of the vector to release the DNA fragments and Tucholski teaches that *Mme*I is a class IIS restriction enzyme capable of cutting 20/18 nucleotides away from its asymmetric recognition sequence, and that it thus would have been obvious to use other class IIS restriction enzyme sites in the adapters of the vectors taught in the method of DNA assembly in the '561 application. This rejection is traversed.

Claim 49 is a dependent claim directed to a method of preparing a vector comprising a ditag of the present invention wherein a particular restriction site is used. The shortcomings of the primary reference have been discussed above relative to the claims from which claim 49 depends, and that discussion is equally applicable to this rejection. There is no motivation to combine the teachings of the cited secondary reference with those of the primary reference, and even if one were to do so, the secondary reference does not compensate for the deficiencies of the primary reference and the combination does not lead one to the presently claimed invention. As the references do not teach or suggest the claimed method for preparing a vector comprising a ditag of the present invention, the references certainly do not teach or suggest such a method in which a particular restriction site is used.

Finally, the examiner has rejected claims 50-52 as unpatentable under 35 U.S.C. §103(a) as obvious over the two references cited in the preceding rejection, taken further in view of GenBank accession number X65305.2. The accession number is for the pGEM-4Z vector. The examiner has asserted that the primary reference provides that appropriate class II restriction enzymes be used, while the secondary reference

teaches *Mme*I as a class IIS restriction enzyme, and the tertiary reference teaches a vector which contains multiple cloning sites and lac operator like that of pWB taught by the primary reference, which is the backbone of the vector used for SE ID NO:18 and can be used to make a vector of similar function to pWB. This rejection is traversed.

As Applicants previously have stated, the purpose for having a vector containing SEQ ID NO:18 is to ensure that the 5' and 3' termini of the same nucleic acid molecule are obtained. This is not a focus in the methods taught in the '561 application primary reference. As no such limitation is disclosed in either the secondary or tertiary references, the combination of these references with the primary reference would not produce a vector as is required in the present invention. There would be no motivation for one of skill in the art to combine the three references cited by the examiner, and, as noted, above, if one were to do so, he would not arrive at the present invention. The resultant product would be very different from that claimed, and the cited references do not render obvious the subject matter of claims 50-52.

Applicants respectfully submit that the claims are in condition for allowance.

Respectfully submitted,

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